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ON-LINE PRECONCENTRATION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF *o*-TOLUENESULPHONAMIDE IN SACCHARIN

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SUMMARY

The on-line preconcentration of *o*-toluenesulphonamide on two commercial reversed-phase liquid chromatography sorbents was examined with regard to the trace analysis of this compound in saccharin. Owing to the low retention of this impurity on the sorbents examined, the entire sorption capacity of precolumn had to be utilised. Supposing that the same sorbent is used for the precolumn and the analytical column, the optimum volume of the precolumn was estimated. The enrichment factor is *ca.* 50, thus making it possible to determine *ca.* 0.1 ppm of *o*-toluenesulphonamide in the sodium salt of saccharin.

INTRODUCTION

On-line preconcentration has become widely used in high-performance liquid chromatographic (HPLC) trace analysis. This technique is based on the adsorption of analytes from a sample solution on to a small amount of a suitable sorbent. A precolumn placed upstream from an analytical column serves for the accommodation of the sorbent. As the precolumn is usually connected to the analytical column by means of a six-port sampling valve, preadsorbed analytes are easily transferred to the analytical column after switching the valve the inject position.

For the analysis of various aqueous samples such as urine, blood plasma or waste water, this technique of on-line preconcentration is used in combination with reversed-phase liquid chromatography (RPLC)[1–4]. Non-polar sorbents such as activated carbons, styrene–divinylbenzene resins and C_{18}-modified silicas are used for the preconcentration. The efficiency of preconcentration therefore depends upon the adsorption coefficient of analyte, which is influenced by the mutual affinity of the analyte and the sorbent. As the compounds to be analysed have to be strongly adsorbed from aqueous solutions, problems may occur when polar analytes are preconcentrated. On the other hand, a rapid desorption is required when the preconcentrated analyte is transferred onto the analytical column.

In this paper, on-line preconcentration was used for the determination of *o*-

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toluenesulphonamide (*o*-TS) in saccharin. As the majority of this artificial sweetener is manufactured by the Remsen–Fahlberg method[5], which includes oxidation of *o*-TS as the last reaction step, this compound is reported to be the main impurity present in food-grade saccharin[6,7].

The content of *o*-TS in commercial samples varies from several ppm up to 0.1%[10]. Although it has been reported recently that saccharin formulations can easily be analysed by RPLC, various extraction and clean-up procedures are required prior to carrying out the chromatography[8–10]. The detection limit of *o*-TS in food-grade saccharin is *ca.* 10 ppm, using ultraviolet (UV) detection at 268 nm[10].

As *o*-TS is adsorbed more than the sodium salt of saccharin (sodium saccharin) on non-polar sorbents, the use of on-line preconcentration seems to be a promising method for enhancing the sensitivity of HPLC analysis. Owing to the relatively high polarity of *o*-TS, the break-through volumes observed on various C_{18}-modified silicas are in the millilitre range of analysed solutions. The preconcentration was therefore carried out in a manner that allowed the highest possible enrichment factor to be reached.

The detection limit depends upon the sorbent used and varies from 0.1 to 1 ppm of *o*-TS in sodium saccharin when UV detection at 268 nm is used. Moreover, solutions of sodium saccharin are analysed directly with good precision with no off-line clean-up procedure being required. Two commercial C_{18}-silicas were tested as sorbents for *o*-TS preconcentrtion and RPLC analysis.

THEORY

If dilute solutions of an analyte A are pumped through a precolumn, break-through curves are obtained as a dependence of the outlet concentration, C_A, on the passed volume, V_s (Fig. 1). It is stressed in the literature[4,11,12] dealing with on-line preconcentration that the volume, V_s, of passed sample should not exceed the break-through volume, V_b. In that case, all the analyte is trapped on the sorbent.

The break-through volume is defined[4] as

$$V_b = V_r - 2\sigma_v \tag{1}$$

where V_r is the retention volume of the analyte A and σ_v is the standard deviation, which is dependent upon the axial dispersion of analyte along the bed of sorbent particles.

Since the standard deviation can be expressed in terms of the precolumn length (L_p), the diameter of the sorbent particles (d_p) and the reduced plate height (h), Eqn. 1 is transformed to a more practical form (Eqn. 2).

$$V_b = V_r(1 - 2\sqrt{hd_p/L_p}) \tag{2}$$

Thus, V_b depends upon h, which is a function of the reduced flow velocity of the sample through the precolumn, the sorbent bed geometry and the rate of mass transfer as given by the Van Deemter equation[13].

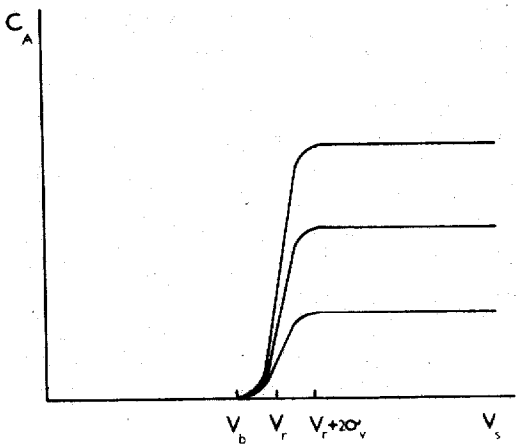

Fig. 1. Break-through curves obtained for various c_A values.

It is evident that the exact value of h is usually unknown for a particular precolumn. Nevertheless, a reduced value, *ca.* 10 could be expected for relatively short and hand-packed precolumns.

The enrichment factor, f, is defined as the ratio between the amount of analyte entering the analytical column after preconcentration and the amount injected without preconcentration. This factor is given by the mass balance of the analyte:

$$f \leqslant V_b/V_0 = (1 + k'_{H_2O})\,(1 - 2\sqrt{hd_p/L}) \tag{3}$$

where V_0 is the dead volume of the precolumn and k'_{H_2O} is the capacity factor of the analyte retained from a water sample. Thus, for low-retained analytes possessing low k'_{H_2O} values and poorly packed and short precolumns, f is relatively low. There are two methods of increasing f for a given k'_{H_2O}. The first is optimisation of the precolumn geometry in order to minimise h; this has been discussed by Werkhoven-Goewie *et al.*[11]. These authors recommend use of narrow-bored, and therefore longer, precolumns which can be packed by the slurry-packing technique. The second method, used by us, is to equilibrate the entire precolumn packing with analyte.

Thus, if the volume of sample passing through the precolumn is greater than $V_r + 2\sigma_v$, the precolumn is completely equilibrated with analyte. At low analyte concentrations, the amount of analyte adsorbed is given by the linear adsorption isotherm which has a slope K_A. The amount of analyte adsorbed in a fully equilibrated precolumn is therefore given by the relationship

$$m_A = V_p\,\rho\,S\,(1 - \varepsilon_T)K_A c_A \tag{4}$$

and is therefore directly proportional to the concentration of analyte, c_A, in the sample. The other variables such as precolumn volume (V_p), adsorption constant (K_A), specific surface (S) and density (ρ) of the sorbent used and the total porosity of the bed (ε_T) are empirical constants.

It must be stressed however that this mode of preconcentration can be used only if the composition of the sample being analysed is practically unchanged in respect of the major components. Under this condition, K_A or k'_{H_2O} is practically constant. In the case of saccharin analysis this can be easily fulfilled when a constant concentration of sodium saccharin is maintained throughout the measurement.

Optimisation of the precolumn volume, V_p, is another problem, as the amount of preadsorbed analyte, which is proportional to V_p, should be as large as possible. On the other hand, the performance of the analytical column decreases with increasing volumes of sample injected. The existence of an optimum volume was anticipated by several authors[1,4,11]; however they did not attempt to estimate its value.

To a first approximation, it is possible to assume that the precolumn, if packed with the same sorbent, is part of the analytical column. Band broadening in the connecting capillaries is not considered, as it has been shown that under appropriate experimental conditions it can be neglected[11].

For a rectangular sample band occupying the initial part of a chromatographic column, a condition limiting the volume of this part can be found in the literature[13–15]. It has been stated by Ván Deemter *et al.*[13] that the efficiency of the chromatographic column is not influenced markedly if the width of the input band does not exceed one quarter of the width of the peak at the output. If the precolumn diameter equals the diameter of the analytical column and the same porosity of bed is supposed, we obtain an approximate condition for the ratio between the length of the precolumn, L_p, and the analytical column, L_c:

$$L_p/L_c \leqslant (1 + k')/2\sqrt{N_c} \tag{5}$$

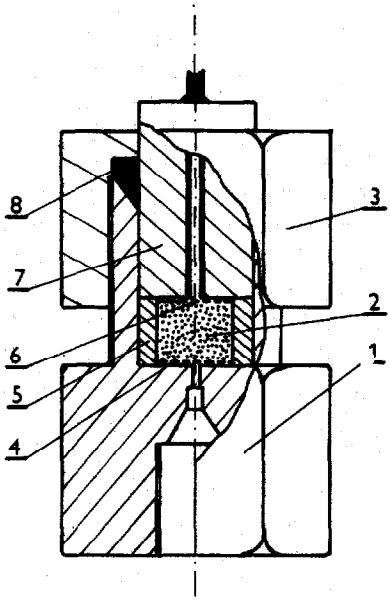

Fig. 2. Schematic diagram of the precolumn: 1 = Swagelok body, 2 = sorbent bed, 3 = Swagelok nut, 4 = outlet mesh, 5 = stainless steel tube (3.5 × 4 mm I.D.), 6 = inlet mesh, 7 = inlet capillary, 8 = ferrule.

This condition is valid only if the number of plates, N_c, of the analytical column is sufficiently high ($N_c > 10^3$).

EXPERIMENTAL

For the preconcentration experiments, the chromatograph consisted of an Altex pump (Model 110 A, Altex, Berkeley, CA, U.S.A.), a sampling valve (Model 70-10, Rheodyne, Berkeley, CA, U.S.A.) and a variable-wavelength UV detector (UVM-4, Development Workshops, Prague, Czechoslovakia). The design of the precolumn connected with the injection valve instead of a sampling loop is shown in Fig. 2. The precolumn is leak-proof up to 300 bar. The sorbent used for preconcentration was hand-packed into the inner space of the precolumn (3.5 × 4 mm I.D.). During the preconcentration step, aqueous samples of sodium saccharin were passed through the precolumn by means of a 1-ml glass syringe.

Stainless steel columns (250 × 4 mm I.D.) were home-packed with Separon Si-C_{18} (10 μm) (Laboratorní přístroje, Prague, Czechoslovakia) and Nucleosil 10, C_{18} (Macherey-Nagel, Düren, F.R.G.). Methanol–aqueous 0.05 *M* sodium nitrate (40:60) was used as the mobile phase at a flow-rate of 1 ml/min.

The samples of food- and technical-grade sodium saccharin as well as the *o*-TS were supplied by Spolana (Neratovice, Czechoslovakia). Aqueous solutions of sodium saccharin, free from *o*-TS, were obtained when 10 ml of food-grade sodium saccharin solution were passed through C_{18} Sep-Pak (Waters Assoc., Milford, MA, U.S.A.); the Sep-Pak cartridge was preconditioned with 5 ml of methanol and 5 ml of distilled water prior to each use. *o*-TS (0.1 g) was dissolved in 5 ml of methanol and diluted with distilled water up to 100 ml. The purified aqueous solutions of sodium saccharin were spiked with known amounts of the *o*-TS solution to prepare standard samples. Each measurement was repeated three times.

RESULTS AND DISCUSSION

The capacity factor, k', of *o*-TS on the analytical column was 0.6 for Nucleosil and 0.9 for Separon. The number of plates, N_c, was *ca.* 3500 ± 150 for the analytical column packed with Nucleosil and 2800 ± 150 for the second sorbent, when samples were injected by means of a 20-μl loop. According to Eqn. 5, the maximum length of the precolumn is 3.5 mm for the Nucleosil column and 4.5 mm for the Separon one. As the actual length of the precolumn used was 3.5 mm, the performance of both columns should not be influenced by the fact that *o*-TS is introduced via the precolumn. When the loop was replaced by the precolumn, the efficiency of both columns was found to be practically unchanged: N_c = 3300 ± 150 for Nucleosil and 2800 ± 200 for Separon.

Werkhoven-Goewie *et al.*[11] compared the efficiency of an analytical column (N_c = 8300) connected to precolumns of various length with 3,5-dichlorophenol as test solute (k' = 1.17). According to Eqn. 5, the maximum length of the sample band is estimated to be *ca* 1.5 mm. For two precolumns with L_p = 1.45 mm, no difference between a loop injection of 10 μl and a preconcentration of 5 ml of sample (V_b = 7 ml) could be measured within the range of experimental error. Nevertheless, the efficiency of the whole system (precolumn–column) decreased markedly to N_c = 7450

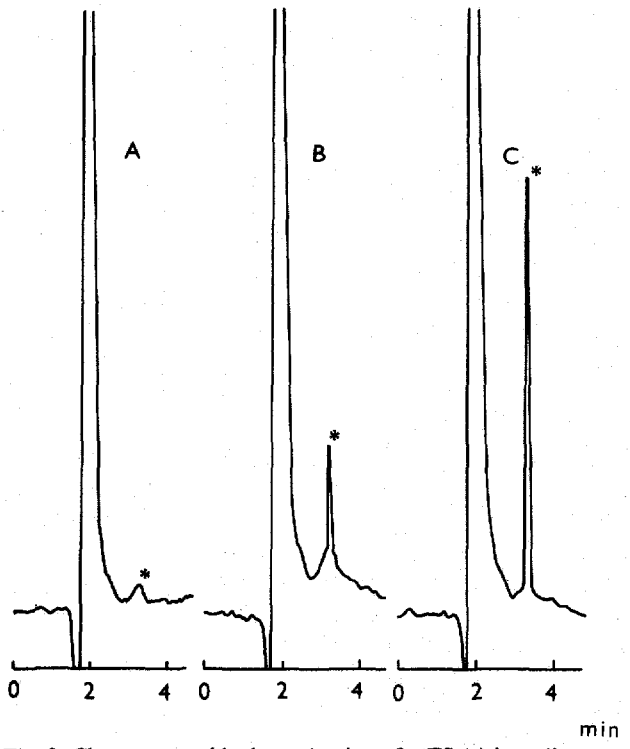

Fig. 3. Chromatographic determination of *o*-TS (*) in sodium saccharin: (A) loop injection of 20 μl, 20 ppm of *o*-TS; (B) loop injection of 20 μl, 200 ppm of *o*-TS; (C) preconcentration on Nucleosil 10, C_{18}, 20 ppm of *o*-TS. Nucleosil 10, C_{18} column (250 × 4 mm I.D.), methanol–aqueous 0.05 *M* sodium nitrate (40:60), flow-rate 1 ml/min, UV detection at 268 nm; concentration of sodium saccharin in injected samples, 10 g/l.

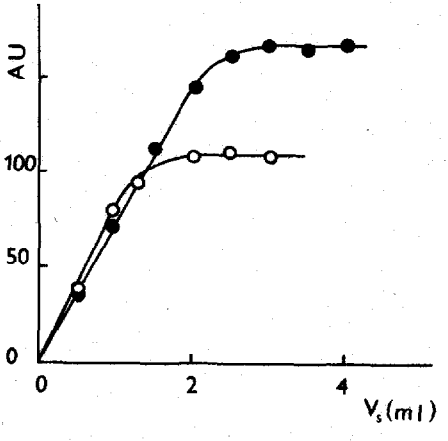

Fig. 4. Dependence of detector response [in arbitrary units (AU)] upon the volume of sample, V_s, passed through the precolumn: ○, Nucleosil 10, C_{18}; ●, Separon Si-C_{18}. Flow-rate of mobile phase, 1 ml/min; detection at 268 nm.

and 7150, respectively. The decrease in the number of plates and the increase in peak asymmetry were probably due to an additional dispersion taking place in the connecting parts. As our columns possessed a much lower efficiency, these effects could not be observed (Fig. 3).

The dependence of detector response upon varying V_s is shown for the both columns in Fig. 4. It is evident that the height of the *o*-TS peak does not depend upon V_s if $V_s \geq V_r + 2\sigma_v$. The break-through volumes of *o*-TS are different for the two sorbents, being *ca.* 1.25 ml for Nucleosil and 2.0 ml for Separon.

Thus, enrichment factors, *f*, *ca.* 45 for Nucleosil and *ca.* 60 for Separon were found when $V_s \geq V_r + 2\sigma_v$. Assuming the number of precolumn plates to be approximately 50, the gain in *f* is *ca.* 30% when compared to the preconcentration with respect to V_b. But the main advantage in this case is that the height of the *o*-TS peak is independent of V_s (Fig. 4). A linear calibration plot obtained for both columns can serve as proof of the validity of Eqn. 4, if the preconcentration takes place under a constant K_A. The reproducibility for repeated analysis ($n = 3$) of the same sample is *ca.* 3% relative standard deviation.

CONCLUSION

It has been shown that under a relatively constant composition of sample solution, the entire adsorption capacity of the preconcentration precolumn can be utilised. This is advantageous for analytes which have only low adsorption on the sorbent during the preconcentration step. The volume of the precolumn can be optimised according to Eqn. 5.

The feasibility of the above method of preconcentration was tested on the HPLC analysis of trace quantities of *o*-toluenesulphonamide in the sodium salt of saccharin. The results show that the detection limit of the impurity can be lowered approximately fifty times (Fig. 3). The technique enables the detection of *ca.* 0.1 ppm of *o*-toluenesulphonamide using an ordinary RPLC column and a UV detector operating at 268 nm.

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